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Full Length Research Paper

Effect of natural antioxidant – *Ocimum gratissimum* in modulating neurodegenerative changes in rats fed with high concentration of lead acetate

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Lead (Pb) poisoning and the role of antioxidants in its pathologies especially their oxidative tendencies on the central nervous system is well documented. The effect of a natural antioxidant-*Ocimum gratissimum (ocimum)* - in modulating neurodegenerative changes in rats experimentally fed with high concentration of Pb acetate was investigated. Standard laboratory methods were employed to determine blood Pb levels and glutathione activity in the homogenized brain tissue of the experimental animals. Rats fed Pb showed significantly (P<0.05) high blood Pb levels (22.86±2.38 µg/dl) relative to the control (10.71±0.92 µg/dl) not fed extract of Ocimum. Significant (P<0.05) reduction of blood Pb levels was observed in rats fed with 10mg/ml extract of *Ocimum* (16.38±2.39 µg/dl) as against levels in Pb-induced rats (22.86±2.38 µg/dl). Reduced glutathione activity was enhanced by the administration of ocimum extract in the experimental rats. Lipid peroxidation due to neurotoxic changes was observed in the brain tissue of rats fed with high concentration of Pb while the neurodegenerative modulating effect of ocimum extract was observed in the group of rats supplemented with the extract. Thus, extracts of this plant after proper standardization could be a good supplement in the management of neurodegenerative changes occasioned by excessive Pb ingestion in children with Pb encephalopathy.

Key words: Aqueous extract, *Ocimum gratissimum*, oxidative stress, neurotoxic, lead acetate.

INTRODUCTION

Various harmful and toxic chemical compounds are formed as bye/intermediate products of normal biochemical reactions in the human system; these bye products are eliminated or detoxified under normal physiological conditions. Free radicals and reactive oxygen species (ROS) are amongst these chemicals and their accumulation when not eliminated by the endogenous system often lead to oxidative stress. Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS include free radicals such as superoxide anion radicals ($.O_2$-), hydroxyl radicals (OH·), and singlet oxygen (Halliwell, 1995). Cells are equipped with different kinds of mechanisms to fight against free radicals and reactive oxygen species and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury (Bergendi et al., 1999). However, accumulation of these chemicals, often lead to deleterious effects in the human body. Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries that is oxidative damage (Jainu and Shyamala, 2005).

Several theories have been proposed as the

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pathophysiological basis of this oxidative damage leading to tissue injuries. Amongst these is that excessive amount of ROS initiate biomolecular oxidation and create oxidative stress which results in numerous diseases and disorders (Halliwell, 1994; Rackova et al., 2007), these diseases include neurodegenerative disorders (Sas et al., 2007). In addition, oxidative stress causes inadvertent enzyme activation (Wiseman and Halliwell, 1996), a phenomenon that has wide implication in the various enzyme-based biochemical reactions in the body. The main focus of this work is to stimulate disturbances in the antioxidant defense mechanisms especially the biochemical equilibrium between reduced and oxidized glutathione by excessive lead ingestion and to determine whether this disturbance has led to neurodegenerative changes in the animal model used.

Reduced glutathione (gamma-glutamylcysteinylglycine (GSH)), a tripeptide with a free thiol group, is a major antioxidant in human tissues that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of hydrogen peroxide and lipid hydroperoxides to water. During this process, GSH becomes oxidized glutathione (GSSG).

The GSSG is then recycled into GSH by glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) as in the equation below. Normally, in the human system, the ratio of GSH/GSSG is >1. However, when mammalian cells are exposed to increased oxidative stress, the ratio of GSH/GSSG will decrease as a consequence of GSSG accumulation.

Measurement of the GSSG level or determination of the GSH/GSSG ratio is therefore a useful indicator of oxidative stress and can be used to monitor the effectiveness of antioxidant intervention strategies.

$$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

A number of natural antioxidants have been employed traditionally to maintain the above delicate balance between GSH and GSSG (Akinmoladun et al., 2007). The commonest amongst them is *O. gratissimum* (also known as scent leaf, sweet basil in English, efinrin ajase in Yoruba, ntong in Efik, aai doya ta gida in Hausa, and nchuanwu in Igbo). Phytochemical studies of extract of the plant have shown that it contains Alkaloids (1.28%), Tannin (0.18%), Saponin (0.22%), Flavonoids (0.04%), and Sterols (0.64%) amongst others (Dike, 2010), and of all these phytochemical compounds, tannins and flavoinoids are known compounds with antioxidant properties. This natural plant available almost ubiquitously in the tropics is being employed in this study to evaluate its antioxidant effects on neurodegenerative changes induced by reactive oxygen species in experimental lead poisoning.

MATERIALS AND METHODS

Animals

Forty healthy adult Wister rats weighing 187 g (averagely) were used. The rats were obtained and kept in the Animal House Unit of Ladoke Akintola University of Technology, Ogbomoso. The rats were allowed to acclimatize for a period of two weeks.

Chemicals

All chemicals used in the study were of analytical reagent grade.

Preparation of aqueous extract

The leaves of *O. gratissimum* were collected in Osogbo, Osun State, Nigeria. The plant was identified and authenticated at Forest Research Institute of Nigeria (FRIN), Jerico, Ibadan, Oyo State, Nigeria. The leaves of *O. gratissimum* were dried at room temperature and pulverized. 40 g of the powdered leaf was dissolved in 400 ml of distilled water and stirred with magnetic stirrer for 2 h at 7000 rpm. The mixture was left for 24 h to ensure total extraction of the residue after which the mixture was filtered with muslin cloth to separate the filtrate and the residue. The filtrate was evaporated to dryness in the oven at 45°C. From the stock of scent leaf extract, 1000% (w/v) solutions were prepared.

Acute toxicity (LD_{50}) study

The lethal dose (LD_{50}) of the plant extract was determined using the method of Lorke (1983). 20 mice were divided into 5 groups of 4 mice each and were given the aqueous extract of the *O. gratissimum* at doses of 312.5, 625, 1250, 2500 and 5000 mg/kg body weight after being fasted for 24 h. They were observed for 24 h for signs of toxicity. At 1250mg/kg dose, motality ratio of 2/4 (50%) was observed. The dose used for this study was selected on this basis which compared favourably with previously performed acute toxicity study in mice.

Lead acetate for induction of lead poisoning

The lead acetate model described by Rader et al. (1981) was used for scheduling the dose regimen. 200 µg of lead acetate/ml oral dose per week was employed for inducing lead poisoning in the adult Wister rats.

Experimental design

The rats were randomly divided into four groups (n = 10). Each group was caged separately. All rats were fed with commercial rat feed and distilled water *ad libitum*. The cages were cleaned daily and food/water changed daily. The commercial rat feed was obtained from TOPFEED, Nigeria.

Group 1 = Negative control: Normal feed and water daily.
Group 2 = Experimental group I: Normal feed and water daily and 200µg lead acetate/ml orally once per week.
Group 3 = Experimental group II: Normal feed and water daily and 10 mg/ml aqueous extract of *O. gratissimum* orally trice per week.
Group 4 = Experimental Group III: Normal feed and water daily, 10 mg/ml aqueous extract of *O. gratissimum* orally trice per week, and 200 µg lead acetate/ml orally once per week. After the

Table 1. Percentage (%) yield of plant leave of *Ocimum gratissimum*

Name of plant	Family	Plant part	Plant extract	% yield
Ocimum gratissimum	Lamiaceae	Leave	Aqueous	4.3

Table 2. The acute toxicity study (ld_{50}) of aqueous extract of *Ocimum gratissimum* in mice.

Name of plant	Family	Plant part	Plant extract	LD_{50} (mg/kg)
Ocimum gratissimum	Lamiaceae	Leave	Aqueous	1250

Table 3. Blood lead concentration, total glutathione, reduced glutathione (GSH), oxidized glutathione (GSSG) levels and GSH/GSSG ratio of the different experimental groups.

Groups	Treatment	Blood lead concentration (µg/dl) Mean ± SD	Total glutathione (µM)	Reduced glutathione (µM) Mean ± SD	Oxidized glutathione (µM) Mean ± SD	GSH/GSSG ratio <1 or >1
Group I	Control-normal	10.71 ± 0.92	7.27	4.93 ± 2.07	2.33 ± 1.52	>1
Group II	Lead-exposed	22.86 ± 2.38	10.55	3.28 ± 2.61	7.27 ± 2.01	<1
Group III	Lead exposed and *O. gratissimum*	16.38 ± 2.39	8.61	6.68 ± 0.20	1.93 ± 1.80	>1
Group IV	*O. gratissimum*	-	7.51	5.74 ± 4.32	1.78 ± 1.11	>1

experimental period of 2 months, the rats were sacrificed by humane killing of experimental animal. The blood samples were collected into heparinised bottles and used for estimation of blood lead concentration. A weighed section of the brain for each rat in each group was homogenized and centrifuged. The supernatant was then used for GSH and GSSG estimation after deproteinization.

Biochemical analysis

Blood lead concentrations were determined using Atomic Absorption Spectrophotometry (AAS) based on the modified method of Hessel (1968). Glutathione measurements were estimated according to the method of Ellman (1959). Histochemical techniques for detection of lipid peroxidation were performed. Lipid peroxidation (n = 4) was based on modified direct Schiff's reaction (Taper et al., 1988).

Statistical analysis

Results of the biochemical estimations are reported as Mean ± SD. Statistical analysis was performed using student's t-test and $P \leq 0.05$ being considered statistically significant.

RESULTS

The plant and plant part and percentage yield of *O. gratissimum* used for this study is presented in Table 1. The 50% lethal dose (LD_{50}) of aqueous extract of *O. gratissimum* in mice is presented in Table 2. The blood lead level, total glutathione, reduced glutathione (GSH), oxidized glutathione (GSSG) levels and GSH/GSSG ratio of the different experimental groups is presented in Table 3. There was a significant ($P<0.05$) mean difference between the blood lead level in lead-exposed rats and normal control rats, and between mean blood lead level in lead-exposed rats and lead-exposed rats receiving aqueous extract of *O. gratissimum*. At $P>0.05$, no significant mean difference existed between GSH level in normal control rats and lead-exposed rats; between lead-exposed rats and lead-exposed rats receiving aqueous extract of *O. gratissimum* as well as between lead exposed rats receiving aqueous extract of *O. gratissimum* and rats receiving the extract only.

There was a significant mean GSSG level between normal control rats and lead-exposed rats, but no significant mean difference existed between mean GSSG level in lead-exposed rats and lead-exposed rats receiving aqueous extract of *O. gratissimum* as well as between lead-exposed rats receiving aqueous extract of *O. gratissimum* and rats receiving the extract only. The GSH/GSSG ratio was >1 in normal control rats, lead-exposed rats receiving aqueous extract of *O. gratissimum* and rats receiving the extract only but <1 in lead-exposed rats. The histochemical result is presented in Table 4. There was no positive reaction in brain section of normal

Table 4. Histochemical results

Groups	Group I	Group II	Group III	Group IV
Treatment	**Control-normal**	**Lead-exposed**	**Lead exposed and *O. gratissimum***	***O. gratissimum***
Brain section	No positive results were obtained	Schiff positive areas presented well circumscribed foci	No positive results were obtained	No positive results were obtained

control rats, lead-exposed rats receiving aqueous extract of *Ocimum gratissimum* and rats receiving the extract only but the lead exposed rats gave a positive reaction which presented well circumscribed foci.

DISCUSSION

Lead poisoning initiates the release of free radical leading to the generation of reactive oxygen species (ROS), including hydro peroxides, singlet oxygen and hydrogen peroxides, and direct depletion of antioxidant reserves (Ercal et al., 2001). One of the effects of lead exposure is on glutathione metabolism by effectively binding and in-activating the glutathione molecule making it unavailable as an antioxidant. As earlier stated, glutathione is one of the major antioxidant of the body which effectively bind free radicals. Studies have assessed the effect of antioxidants as chelating agents and their use in conjunction with lead chelators in reducing lead tissue burden (Gurer et al., 1998), oxidized glutathione (GSSG) levels, and in the prevention of free radical damage variously (Flora et al. 2003).

However, the studies that assessed the effect of antioxidant as chelating agents did not show that antioxidants acted as effectively as conventional chelating agents in reducing lead tissue burden (Gurer et al., 1998; Flora et al., 2003). For example, in a study of lead-exposed rats treated with N-acetylcysteine (NAC), reduction of blood lead from a baseline of 34.8 to 25.3 µg/dl (27.3% reduction) after one week of treatment was observed whereas meso-2,3-dimercaptosuccinic acid (DMSA; Succimer – a chelating agent used in pediatric and adult treatment of lead toxicity) alone reduced blood lead levels to 2 to 5 µg/dl (Gurer et al., 1998) over the same period of administration (over 75% reduction). The search for drugs/chemicals that will prevent/modulate the toxicity of lead and other toxic metals especially their antioxidant activity is continuous.

In the present study, the effect of natural antioxidant-*O. gratissimum* in modulating the attendant neurode-generative changes in rats experimentally fed with high concentration of lead acetate was investigated. It was found that levels of lead in blood of normal control group was significantly ($p<0.05$) less (10.71±0.92 µg/dl); ($p<0.05$) than among lead-exposed groups. Also, a significant reduction was observed in the group of rats exposed to lead and aqueous extract of *O. gratissimum* relative to blood lead level of 22.86±2.38 µg/dl in lead-exposed only. Reduced glutathione (GSH) was preserved and enhanced by the oral administration of the plant extract in the experimental rats as observed in the increased GSH level of 6.68±0.20µM in the group of rats exposed to lead and aqueous extract of *O. gratissimum* relative to GSH level of 3.78±2.61 µM; $p>0.05$ in lead-exposed rats and GSH level of 5.74±4.23 µM in rats receiving aqueous extract of *O. gratissimum* compared to GSH level of 4.93±2.07 µM; $p>0.05$ in normal control group.

Depletion of GSH and production of ROS in brain tissue of lead-exposed rats resulted in significant GSSG production (7.27±2.01 µM) compared to GSSG level of 2.33±1.52 µM in normal control group and consequently a reduction in the GSH/GSSG ratio 0.45 (<1). Normally, in mammals, the ratio of GSH/GSSG is >1 and when mammalian cells are exposed to increased oxidative stress, the ratio of GSH/GSSG decreases as a consequence of GSSG accumulation. In this study, lead-exposed rats receiving aqueous extract of *O. gratissimum* had GSH/GSSG ratio of >1 (3.45) which most likely was due to free radical scavenging activity of the extract with consequent reduction in GSSG level (1.93±1.80 µM) compared to lead-exposed rats not receiving the extract. It has been stated earlier that lead poisoning initiates the release of free radical leading to the generation of reactive oxygen species.

One of the main target substrates for these oxygen radicals include polyunsaturated fatty acids in the membrane phospholipids whose modification result in disorganization of cell framework and function (Todorova et al., 2005). The consequence of these reactions (peroxidation process) is the appearance of aldehydes in cells which in normal conditions do not contain these compounds (Esterbauer, 1982). Detection of one of these aldehydes, namely malondialdehyde serves as a marker of lipid peroxidation and the presence of oxidative stress respectively. Histochemical detection of aldehydes gave useful information on tissue and organ intoxication. The appearance of these aldehydes precedes distinct morphological alterations detectable by histochemical techniques. Malondialdehyde formed by lipid peroxidation initiated by free radicals was demonstrated by direct Schiff's reaction.

Schiff's positive areas presented well circumscribed foci as was observed in brain section of lead-exposed rats. No positive reactions were observed in other

groups.

Conclusion

The search for a solution to the problem of lead toxicity especially in the vulnerable groups is ongoing and the current work on antioxidant status could bring the necessary solution. The need for solution to the problem of lead toxicity is underscored by its near ubiquitous nature and almost compelling use in most industries and homes. That lead affects mammalian system by directly lowering antioxidant reserves and generating ROS resulting in oxidative damage is not in doubt. However, some qualitative inferences may be drawn from the present study: aqueous extract of *O. gratissimum* lowers or interrupt the damaging effects of lead and reduces blood lead concentration – an indication of the synergism that exist between the antioxidant and metal chelating properties of the plant extract.

Therefore, if properly standardized, the extract of this plant could be a good supplement in the management of neurodegenerative changes occasioned by excessive lead ingestion in children with lead encephalopathy and could be adopted as a preventive/protective agent against lead poisoning especially in the vulnerable group.

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